

A2
insert

BLACKCURRANT PROMOTERS AND GENES

The present invention relates to transgenic plant production and the expression of gene sequences introduced by genetic transformation procedures. In particular the present invention relates to blackcurrant (*Ribes nigrum* L.) fruit-specific gene promoters and their use in the expression of nucleic acid sequences in transgenic fruit.

Studies on the molecular basis of fruit ripening have concentrated on species whose fruit exhibit a climacteric pattern of ripening, for example tomato, avocado, apple, kiwifruit, peach and mango. Ripening in the fruit from these species is accompanied by a burst in the rate of respiration and a generally large increase in the rate of biosynthesis of the plant growth regulator, ethylene.

Non-climacteric fruit have a considerably different ripening mechanism. Examples of non-climacteric fruit are blueberry, cucumber, grape, orange and strawberry.

Fruit ripening is an important area of scientific research with particular attention being paid to high value fruits such as tomato, kiwifruit and avocado. In the tomato some of the genes involved in the ripening process have been isolated and characterised, for example the gene for polygalacturonase, an enzyme which acts on cell wall pectin. The level of expression of the polygalacturonase gene has been down-regulated in transgenic tomato fruit resulting in increased fruit firmness and consequently extended storage life (Schuch *et al.*, 1991).

In contrast, less is known about the molecular basis of fruit ripening in non-climacteric fruit. In the work leading to the present invention we have found from measurements of respiration rate that blackcurrant fruit do not exhibit a respiratory climacteric during ripening and that ripe fruit produce very low levels of ethylene, hence blackcurrant can be classed as a non-climacteric fruit.

The blackcurrant is the most widely grown bush fruit in Europe, valued particularly for its high content of ascorbic acid and anthocyanin pigments. Areas for potential improvement in blackcurrants include enhancing pigment levels, aroma, flavour, texture, nutritional values (e.g. vitamin content), storage life,

weather resistance, pest or pesticide resistance and manipulating sugar, soluble solids or acid levels in the fruit.

Plants with novel/improved characteristics can be produced by introducing genes or DNA sequences from the same or a different organism. Many examples 5 are now in the literature of plant DNA sequences which have been used to drive the expression of foreign genes in plants. In most instances the regions adjacent to the 5' terminus of the coding regions of genes have been used in gene constructs. These regions are referred to as promoter sequences. In order to produce novel 10 phenotypes it is necessary to have active expression of the introduced DNA sequence by cloning the sequence downstream of a promoter sequence active in plant tissue. These promoters may be derived from plant DNA or from other sources e.g. viruses. In most cases sequences up to 500-1000 bases are sufficient to allow for the regulated expression of foreign genes. However sequences longer than 15 1 kb may have useful features which permit high levels of gene expression in transgenic plants. Examples of fruit-specific promoters isolated from climacteric fruit such as tomato include the 2All promoter, and the polygalacturonase gene promoter.

Of considerable importance to the development of genetically improved blackcurrants is the finding in the work of the present invention that blackcurrant is 20 in fact a non-climacteric fruit.

Promoters can vary in the level of expression and in the tissue-specific or developmental stage-specific pattern of expression that they drive. Some promoters are expressed in a tissue-specific or developmental stage-specific manner whereas others are expressed in each and every cell and are called constitutive promoters.

25 The most widely used constitutive promoters are the Cauliflower Mosaic Virus (CaMV) 35S promoter, nopaline synthetase (*nos*) and the octopine synthetase (*ocs*) promoters. Due to the different molecular mechanisms of ripening between climacteric and non-climacteric fruit it is hardly appropriate to use fruit-specific promoters isolated from climacteric fruit such as tomato (e.g. the 2All promoter or 30 the polygalacturonase gene) in non-climacteric fruit.

Climacteric fruit-specific promoters therefore may not be suitable for many potential biotechnological applications for the improvement of non-climacteric fruit

such as the blackcurrant which ideally require high levels of fruit-specific expression. In the case of the commonly used constitutive promoters, they have the disadvantage that they drive expression at high levels in all or nearly all cell types and throughout the development of the plant. Expression of the introduced gene or 5 DNA sequence driven by a constitutive promoter can have a deleterious effect on normal plant development. Additionally, the commonly used constitutive promoters are derived from plant infectious agents such as plant viruses or *Agrobacterium*, a soil-borne infectious bacteria. The source of these promoters is a cause for concern in risk assessment of transgenic plant production.

10 Accordingly, the present invention provides promoters and a process for obtaining promoters capable of driving fruit-specific expression of DNA sequences in transgenic blackcurrant and other non-climacteric fruit. The process is as defined in claim 1 and the promoters as defined in claim 2. Preferably the promoter comprises the sequence of nucleic acid bases in Figure 9 or IDSEQ 11 herein 15 designated the RIBI promoter or in IDSEQ 14 herein designated the RIB 7 promoter. No previous promoters have been reported to be suitable to drive fruit-specific expression in blackcurrant and other non-climacteric fruit.

One advantage of the present invention is that because of the developmental 20 stage specificity of the expression ie. it offers high level expression in fruit and only very low levels in other tissues, there is a reduced chance that the introduced DNA sequences will have an adverse effect on normal plant development.

The promoters of the present invention also have the advantage over some constitutive promoters in that they are naturally occurring plant gene sequences derived from blackcurrants, ie. a plant that is consumed by humans and not from 25 plant pests or other infectious agents; this overcomes objections to the use of such sequences due to potential recombination.

The isolation and characterisation of blackcurrant fruit-specific gene promoters and how they can be used to drive the expression of genes of interest in plants is given below and in the following examples. This description is purely for 30 the purpose of illustrating the invention. It should be noted that the gene promoter may function in a similar (that is, fruit-specific) manner in other related species of non-climacteric fruit, in particular other *Ribes* species.

Promoters for use in the invention may be isolated from genomic libraries by the use of cDNA probes. The cDNA clones of genes highly expressed specifically in ripe blackcurrant fruit were obtained by differentially screening a cDNA library constructed from mRNA isolated from ripening blackcurrant fruit.

5 In a further aspect of the invention there is also provided cDNA for genes which exhibit differential expression in fruit during the ripening period of fruit development. In particular the cDNA is identified herein as pRIB1, pRIB3, pRIB5, pRIB6 and pRIB7.

10 The promoters of the present invention can be used to control the expression of one or more genes in non-climacteric and/or climacteric fruit. Preferably the non-climacteric fruit is the blackcurrant. Suitably the genes are novel/exogenous.

15 According to the present invention we also provide the use of promoters of the present invention in the transformation of plant cells to control the expression of one or more genes in non-climacteric/climacteric fruit.

In a further aspect of the invention there are provided novel plant cells and plants transformed using the promoter according to the present invention. Preferably the plants or seeds are blackcurrants.

20 According to the present invention, plant cells may be transformed using promoters of the invention using a variety of known transformation methods such as *Agrobacterium* - mediated or other vector- mediated transformation methods or physical transformation methods such as biolistics, chemical or electrical transfection or micro-injection.

25 In particular the RIB1 or RIB 7 promoter regions are suitable for incorporation into plasmid vectors designed for general use in construct production in *E. coli*, and for use in stable, *Agrobacterium*-mediated transformation (Bevan, 1984) and in transient transformation (Fromm *et al.*, 1985) or stable, physical transformation methods (Klein *et al.*, 1987). DNA sequences which one wishes to have expressed only in the fruit of transgenic blackcurrants and possibly other 30 non-climacteric soft fruit can be inserted downstream of the promoter region of the blackcurrant RIB1 or RIB 7 gene, prior to introduction into plant cells or production of transgenic plants.

The transformed cells may then, in suitable cases, be regenerated into whole plants in which the new nuclear material is stably incorporated into the genome.

Examples of genetically modified plants according to the invention include as well as blackcurrants, fruits such as blueberry, cucumber, grape, orange and 5 strawberry. Plants produced by the process of the invention may contain more than one recombinant gene. In order to prepare RNA suitable for a cDNA library construction, an improved method for the RNA extraction was developed as the available methods were found not to be applicable to blackcurrent fruit. The problems in working with blackcurrant tissue include the combination of the high 10 levels of phenolic compounds and polysaccharides and the high acidity of berry extracts.

Accordingly in a further aspect of the present invention there is provided a method of extracting nucleic acid in particular RNA from blackcurrant fruit. One known method for grape berries (Tesniere & Vayda, 1991) was found to be unable 15 to yield large quantities of good quality RNA from blackcurrant fruit which was not contaminated with coloured substances. This method was the basis for the modified method for the extraction of RNA from blackcurrant fruit.

Two key modifications were the method of tissue homogenisation and the inclusion of 8.5% (w/v) insoluble polyvinylpolypyrrolidone (PVPP) in the 20 homogenisation buffer. The use of PVPP resulted in the removal of pigment from the fruit pulp at the start of the extraction procedure producing a clear final RNA pellet. Pulping fruit in the homogenisation buffer rather than grinding frozen fruit in a fine powder in liquid nitrogen and then adding the buffer was a less harsh method 25 of tissue maceration and resulted in less disruption of cells and a reduction in the amount of gelatinous material. Pulping also reduced the problem of extracting large amounts of seed as well as fruit RNA which otherwise occurred during grinding in liquid nitrogen. Each fruit can frequently contain over twenty seeds and these are impossible to manually extract quickly enough to prevent the expression and 30 subsequent isolation of wound-induced mRNA's from the fruit. In ripe fruit the problem can be solved using a juicerator (Acme). This macerates the fruit tissue to a pulp which can be collected and retains the seed and large pieces of skin material.

Unripe fruit (i.e. green or green/red) were too hard to be pulped using this method so a coffee grinder was used instead.

The average yield of total RNA using this method is 15-20 µg RNA per g fresh weight of fruit, for each stage of ripening investigated. The ratio of 5 A_{260}/A_{280} nm was between 1.8-2.0. The yield was the same whether RNA was extracted from the pulp on the day of fruit harvest or whether the pulp was stored at -80 °C, defrosted and subsequently used in an extraction. This implies that the RNA remains stable in the pulp. The yields are similar to those obtained from other fruit tissues e.g. apples (13 µg RNA per g fresh weight Lay-Yee et al., 1990) and 10 peaches (12-15 µg RNA per g fresh weight, Callahan *et al.*, 1989).

Denaturing agarose gel electrophoresis revealed that two ribosomal RNA bands were clearly visible suggesting that the RNA extracted using this new procedure was undegraded. In addition the RNA isolated from the fruit was capable of directing the synthesis of polypeptides as demonstrated by *in vitro* translation 15 using a wheat germ lysate system. Polypeptides of up to approximately 80 kD were synthesised and the incorporation of ^{35}S - methionine into TCA precipitable products was about 30 times higher than background values when 20 µg of total RNA were used compared with the minus RNA control.

The new extraction method described below in Example 2 allowed for the 20 first time the extraction of RNA from blackcurrant fruit. This RNA has been shown to be biologically active, as demonstrated by *in vitro* translation results. In addition this RNA has been used to construct a cDNA library from an early ripening stage (Example 4 below). The cDNA library contained approx. 6.6×10^6 primary clones with an average insert size of 900 base pairs. Differential screening 25 of 10,000 clones has resulted in the isolation of 5 clones which show an increase in expression during ripening.

The invention will be described further with reference to the following figures, in which;

Figure 1 shows the results of an RNA blot analysis of total RNA isolated 30 from blackcurrant (cv Ben Alder);

Figure 2 shows the results of a DNA blot analysis;

Figure 3 shows the nucleotide sequence of the pRIB1 cDNA clone (IDSEQ 1);

Figure 4 shows the deduced amino acid sequence encoded by pRIB1 (IDSEQ 2);

5 Figure 5 shows the nucleotide and predicted amino acid sequence of pRIB3 (IDSEQ 3 and 4 respectively);

Figure 6 shows the nucleotide and predicted amino acid sequence of pRIB 5 (IDSEQ 5 and 6 respectively);

10 Figure 7 shows the nucleotide and predicted amino acid sequence of pRIB 6 (IDSEQ 7 and 8 respectively);

Figure 8 shows the nucleotide and predicted amino acid sequence of pRIB 7 (IDSEQ 9 and 10 respectively);

Figure 9 shows the nucleotide sequence of the RIB1 promoter up to the transcription start site (IDSEQ 11), and

15 *A* ^{Figure 10} ~~Figure 10~~ shows the RIB1 gene sequence (IDSEQ 12) and the deduced amino acid sequence (IDSEQ 13). The transcription start site was located by primer extension analysis and this C residue in position 1797 is indicated in bold type and underlined in the figure.

20 **EXAMPLES**

Unless indicated otherwise the methods and standard techniques used below are as given in Sambrook *et al* (1989).

EXAMPLE 1 - Pigment and respiratory analysis

25 *1.1 Plant material*

Fruit, leaves and stems were harvested from blackcurrant (*Ribes nigrum* L. cv. Ben Alder) plants grown in experimental field plots at the Scottish Crop Research Institute, Invergowrie, Dundee, UK. Blackcurrant tissues were harvested and frozen immediately in liquid nitrogen. Thereafter, tissues were stored at -80°C prior to 30 analysis. Roots, leaves and stems were harvested from either one year old plants that had not yet borne fruit or from two-year-old plants that were producing fruit. Fruits

were harvested at five stages of ripening as determined by fruit colour (designated green, green/red, red/green, red and black).

1.2 Determination of fruit anthocyanin content

Blackcurrant fruit (0.5 g FWt) was ground to fine powder in liquid nitrogen
5 and extracted with 1 ml of methanol containing 1% (v/v) trifluoroacetic acid. After centrifugation (16000 g, 10 min) the pellet was re-extracted with a further 1 ml of methanol/trifluoroacetic acid. The absorbance of the combined extracts at 518 nm was determined spectrophotometrically. Anthocyanin concentration in the extracts was estimated by comparison with a standard curve produced using the artificial pigment,
10 amaranth (trisodium 3-hydroxy-4-(4-sulphonato-1-naphthylazo)naphthalene-2, 7-disulphonate).

1.3 Ethylene and CO₂ determinations

The rate of ethylene and CO₂ evolution from harvested blackcurrant fruit was determined using a Hewlett Packard 5890A gas chromatograph. Blackcurrant fruit
15 were placed in gas-tight jars and incubated at 15°C for up to 24 h. Sampling was carried out using a gas-tight syringe. For CO₂ determinations, the gas chromatograph was fitted with a thermal conductivity detector and a Porapak Q column (2 mm internal diameter, 1.85 M length) maintained at 50°C. A flow rate of 20 cm³ min⁻¹ was set for the carrier gas (helium) and the peaks were integrated on a Spectra-Physics
20 integrator (San Jose, California, USA). The chromatograph was calibrated with injections of 1 ml samples of 1% CO₂ (Phase Separations Ltd, Clwyd, Wales, UK). For ethylene measurements, the gas chromatograph was fitted with a flame ionization detector and a Porapak R column (2 mm internal diameter, 1.85 M length) maintained at 80°C. The flow rate of carrier gas (helium) was 50 cm³ min⁻¹ and the system was
25 calibrated by injecting 1 ml samples of ethylene gas at a concentration of 91 ppm (Phase Separations Ltd, Clwyd, Wales, UK). All peaks were integrated using a Hewlett-Packard 3390A integrator.

Results

30 Rate of ethylene and carbon dioxide production by blackcurrant fruit

Very low levels of ethylene were produced by fruit from all stages of ripening (the level of ethylene from green, green/red and red/green fruit was below the

detection limit of the gas chromatograph (approximately 0.1 ppm)). As an indication of the rate of respiration of the ripening fruit, the rate of CO₂ production was determined. There was no burst in respiration rate as the fruit ripened. In fact, the highest rate of CO₂ production was produced by green fruit. In the later ripening 5 stages, the level was approximately 20% lower than in the green fruit and remained constant as the fruit ripened from the green/red to the black stage.

EXAMPLE 2 - RNA Extraction

RNA was extracted from Ben Alder fruit at five ripening stages, and from 10 leaf, root and stem material from fruited and non-fruited Ben Alder plants.

Glassware was baked at 180°C for 12 h and plasticware and Miracloth (Calbiochem) were autoclaved prior to use. Solutions were prepared from stocks by dilution in sterile DEPC-treated (diethyl pyrocarbonate) distilled water before autoclaving. Unless otherwise stated, the procedures were carried out at 4°C. 15 Freshly harvested berries were weighed into 50 g portions and stored on ice. Leaf, root and stem material was harvested, rapidly frozen in liquid nitrogen and stored at -80°C until required. Fruit (50 g) was pulped with 100 ml of homogenisation buffer (200 mM Tris.HCl pH 8.5, 300 mM LiCl, 10 mM Na₂EDTA, 1% (w/v) sodium deoxycholate, 1.5% (w/v) sodium dodecyl sulphate, 8.5% (w/v) insoluble 20 polyvinylpolypyrrolidone (PVPP), 1% (v/v) Nonidet P-40, 1 mM aurintricarboxylic acid, 5 mM thiourea, and 10 mM dithiothreitol (the last three components were added as solids after autoclaving)) in a domestic coffee grinder for 45 s. Leaves, roots and stems were ground to a fine powder in a sterile pestle and mortar, with a little sand (previously baked at 180°C for 12 h) in liquid nitrogen and 5 vol of 25 homogenisation buffer (containing 4% PVPP instead of 8.5%) was added per gramme of tissue. The viscous material was poured into sterile 50 ml tubes. If not required for immediate use, the fruit pulp was frozen in liquid nitrogen and stored at -80°C.

Frozen fruit pulp was defrosted rapidly in a microwave oven prior to use in 30 the extraction. To proceed with the extraction, the homogenate was diluted 1:1 with sterile water and mixed well. 20 ml of diluted homogenate was placed in a 50 ml Oak Ridge-type centrifuge tube containing 15 ml homogenisation buffer and

shaken. The tubes were placed in a waterbath at 65°C for 10 min, with occasional mixing, and then centrifuged at 12,000 x g for 30 min at 4°C. The supernatant was filtered through two layers of Miracloth and collected in an Oak Ridge-type centrifuge tube and solid CsCl was dissolved in the supernatant to a final 5 concentration of 0.2 g CsCl per ml of filtered extract. The extract was gently layered onto a 10 ml cushion of 5.7 M CsCl containing 10 mM Tris.HCl pH 7.5 and 10 mM Na₂EDTA, in a Beckman 50 ml ultracentrifuge tube and centrifuged at 100,000 x g for 20 h at 20°C. After centrifugation, the supernatant was carefully removed with a syringe and discarded. The RNA pellet remained at the bottom of 10 the tube.

The pellet was washed with 5 ml of ice-cold 70% ethanol, centrifuged at 10,000 x g for 10 min at 4°C and the tubes inverted to allow the pellet to dry. The RNA was resuspended in a total of 1 ml of sterile distilled water and transferred to a sterile microfuge tube. 200 µl of 3 M LiCl (0.5 M final concentration) and 2.5 15 ml of 95% ethanol was added to precipitate the RNA (overnight at -20°C).

RNA was recovered by centrifugation at 16,000 x g for 30 min at 4°C, and the pellet was washed three times with 0.5 ml 2.5 M sodium acetate (pH 5.5). Following centrifugation at 16,000 x g for 15 min at 4°C and removal of the supernatant, the pellet was resuspended in 100 µl of sterile distilled water. Ethanol 20 (95%) was slowly added to a final concentration of 30% (v/v) of the total and the tube vortexed briefly. After centrifugation at 16,000 x g for 2 min at 4°C the supernatant containing the RNA was transferred to a fresh microfuge tube and precipitated by the addition of 0.1 vol sodium acetate pH 5.2 and 3 vol ethanol and incubation at -20°C overnight. The RNA was recovered by centrifugation at 16,000 25 x g for 30 min at 4°C, the pellet washed in 0.5 ml 70% ethanol and allowed to dry before it was suspended in sterile water.

EXAMPLE 3 -RNA analysis

Total RNA was extracted from blackcurrant tissues as described above in 30 Example 2. Steady-state transcript levels were determined by RNA blot analysis. Total RNA (15 µg/track) was separated electrophoretically under denaturing conditions and transferred by capillary action onto Hybond-N membranes

(Amersham) as recommended by the manufacturer. Blots were probed with ^{32}P labelled cDNA inserts isolated from cDNA clones following restriction endonuclease digestion. Inserts were separated by electrophoresis through agarose gels and purified by electroelution. After hybridisation for 16-24 h at 42°C in 50% formamide, filters 5 were washed sequentially in 2 x SSC, 0.5% SDS followed by 2 x SSC, 0.1% SDS and then 0.1% x SSC, 0.1% SDS for 20 min per wash at 52°C prior to exposure to X-ray film at -70°C for between 24 and 96 h. Transcript size was determined by comparison of electrophoretic mobility with RNA markers of known sizes (Life Technologies). The intensity of the hybridisation signal was determined by densitometry using a 10 Millipore Bio-Imager (Millipore, Michigan, USA).

Figure 1 shows the results of one RNA blot analysis. Total RNA was isolated from blackcurrant (cv. Ben Alder) leaves (L), stems (S) and roots (R) from plants that had borne fruit and from those that had not, and from fruit at five ripening stages (G = green; GR = green/red; R/G = red/green; R = red; B = black). 15 Total RNA (20 μg per lane) was analysed by electrophoresis through a 1.2% denaturing agarose gel, blotted onto nylon membrane and hybridised with a labelled probe prepared to pRIB1, using standard techniques.

EXAMPLE 4 - cDNA clone isolation and analysis

20 A cDNA library was constructed from polyadenylated RNA (7 μg) extracted from green/red blackcurrant fruit. Polyadenylated RNA was prepared by affinity chromatography using oligo d(T) cellulose (Life Technologies). Double stranded cDNA was synthesised and directionally ligated into *Eco*RI/*Xho*I digested lambda Zap arms using a Uni-Zap XR vector kit (Stratagene). The library was packaged using an 25 *in vitro* kit (Stratagene) and plated on the XL1-Blue strain of *E. coli* (Stratagene).

Differential gene expression during ripening

The cDNA library was screened with ^{32}P labelled cDNA from green fruit and green/red fruit. By differentially screening a total of 10,000 plaques, five were found to be differentially expressed between these stages. The *in vivo* excision protocol of 30 Stratagene with the R408 helper phage was used to rescue putative ripening-related cDNAs in pBluescript SK (-) plasmids. The plasmids were purified using Qiagen columns (Qiagen Ltd., Dorking, UK). Steady-state expression levels of the

corresponding genes (designated RIB1, RIB3, RIB5, RIB6 and RIB7) were determined by RNA blot analysis. The intensities of the hybridisation signals were determined by densitometry. For all clones, very low or negligible levels of expression could be detected in the green fruit and the highest levels of expression 5 were detected in black, fully ripe fruit. In the quantitative densitometric analysis therefore, steady-state transcript levels are expressed relative to the level in black fruit. In order to demonstrate equal loading and transfer of RNA during this analysis, filters were stripped and hybridised with a potato 25S ribosomal RNA probe. An equivalent hybridisation signal was detected for RNA extracted from tissue at all 10 stages (data not shown).

Expression in other blackcurrant tissues

Steady-state expression levels of the RIB genes were also determined in leaves, stems and roots of blackcurrant plants that had borne fruit and from those that had not. A variety of expression patterns were observed. For example, the expression 15 of RIB1 and RIB7 was confined largely to fruit. RIB3, RIB5 and RIB 6 expression however was less specific to fruit and relatively high expression levels could be detected in some of the other plant tissues that were tested. The expression level of some of the clones was different depending on whether the blackcurrant plants had produced fruit or not. For example, the expression level of RIB5 was higher in plants 20 that had never produced fruit compared with tissues from plants that had.

The clone pRIB1 hybridised to cDNA probes prepared from mRNA from ripe fruit but not to cDNA probes prepared from green, unripe fruit. Using the cloned pRIB 1 cDNA as a probe, a blackcurrant (cv. Ben Alder) genomic library constructed in λ Fix II (custom synthesised by Stratagene Ltd, Cambridge, UK) was 25 screened using standard techniques (Sambrook *et al.*, 1989). A genomic clone corresponding to the cDNA clone was isolated and the blackcurrant RIB1 genomic clone was plaque purified. DNA was prepared and fragments subcloned into plasmid vectors by standard procedures (Sambrook *et al.*, 1989). The RIB1 genomic clone contained an insert of 18 kilobase pairs (kbp) from which the 30 relevant sub-fragments were cloned into plasmid vectors. One subclone contains approximately 3 kbp of gene sequence (two exons and one intron) including

approximately 1.8 kbp of 5' flanking sequence which contains the blackcurrant RIB1 promoter region.

RNA blot analysis (Sambrook *et al.*, 1989) of blackcurrant tissues indicates that the gene is highly expressed in ripe blackcurrant fruit and expressed at 5 negligible levels in other tissues of the blackcurrant plant (Figure 1). Therefore this promoter region will be suitable to drive the expression of any piece of DNA cloned downstream of it (that is, following the 3' terminus of the promoter region) in ripening fruit but not in unripe fruit.

10 A positive genomic clone corresponding to the RIB 7 cDNA (RIB 7) was isolated from the blackcurrant (*Ribes nigrum* L., cv. Ben Alder) genomic library and sub-cloned using the same techniques as for RIB 1. Two adjacent sub-clones (as determined by PCR) were sequenced and the RIB7 gene is contained within this sequence.

15

DNA sequence analysis

Plasmid DNA for sequencing was prepared using Qiagen columns. DNA sequence was obtained from both strands of alkaline denatured plasmid by manual dideoxysequencing using Sequenase version 2.0 (United States Biochemical Corporation) or by automated sequencing using an AB1 373 automated sequencer. 20 DNA sequences were compiled and compared using the sequence analysis software and databases available on the SEQNET Computational Molecular Biology facility at SERC Daresbury Laboratory, UK.

Genomic DNA isolation and Southern analysis

25 Genomic DNA was isolated from the leaves of three blackcurrant cultivars (Ben Alder, Ben Sarek and Baldwin), Tayberries (*Rubus loganobaccus*) and raspberries (*Rubus idaeus* cv. Glen Moy). Leaves (1 g FWt) were ground to a fine powder in liquid nitrogen. 2.5 ml buffer containing 2% (w/v) CTAB, 100 mM Tris.HCl pH 8.0, 1.4 M NaCl, 20 mM Na₂EDTA, 0.1% (w/v) DTT at 65°C was 30 added and mixed gently prior to the addition of 0.1 g Polyclar AT (BDH). After a 30 min incubation at 65°C, 7.5 ml of chloroform:isoamyl alcohol (24:1 [v/v]) was added and gently mixed. Following centrifugation (5000 g, 5 min) the aqueous phase was

removed and mixed with an equal volume of propan-2-ol. After a 15 min incubation at room temperature, nucleic acids were pelleted by centrifugation (10000 g, 15 min). The air-dried pellet was resuspended in 0.85 ml water before the addition of 50 μ l 1M KAc, pH 5.5, 20 μ l of 0.5 M Na₂EDTA, 50 μ l Caylase (10 mg/ml [Cayla, Toulouse, France]), 1 μ l RNase A (10 mg/ml [Sigma]) and 29 μ l water. The mixture was 5 incubated for 14 h at 37°C. 50 μ l of 1 M Tris.HCl (pH 8.0) was then added to the solution prior to extraction with one volume of chloroform:IAA (24:1 [v/v]). Genomic DNA was precipitated with three volumes of ethanol, washed with 70% ethanol, air dried and finally resuspended in TE buffer (pH 8.0).

10 5 μ g of each DNA sample was digested separately with the restriction endonucleases *Eco*RI, *Bam*HI and *Hind*III and resolved by electrophoresis on 0.8% (w/v) agarose gels. DNA was transferred under vacuum to Hybond N membranes (Amersham) and hybridised with the ³²P labelled inserts of the pRIB 1 clone, prepared as above. Filters were washed at high stringency (0.1 x SSC, 0.1% SDS at 15 65°C) and exposed to X-ray film for 24-72 h at -70°C with intensifying screens. Figure 2 shows the results of one DNA blot analysis : Genomic DNA (5 μ g per lane) from the blackcurrant cultivars Ben Alder (lane 1), Ben Sarek (lane 2) and Baldwin (lane 3), Tayberry (lane 4) and the raspberry cultivar Glen Moy (lane 5), was digested with either of the restriction endonucleases *Eco*RI, *Bam*HI or *Hind*III, and 20 fractionated on an 0.8% (w/v) agarose gel. The DNA was blotted onto nylon membrane hybridised with a labelled probe prepared to pRIB1, using standard techniques (Sambrook *et al.*, 1989).

Results

Sequence analysis of the pRIB clones

25 pRIB 1

The size of the insert in pRIB1 is 882 base pairs, similar to that expected from the estimate of transcript size. A potential long open reading frame was identified from nucleotide position 3 to the TAA termination codon at position 489. A 30 translation start codon is not present in this ORF indicating that the 5' portion of the cDNA is absent. A polyadenylation signal was identified in the cDNA sequence. Comparison of the deduced amino acid sequence of this ORF and the nucleotide sequence of the cDNA did not reveal any significant sequence similarity to other

sequences in the European Molecular Biology Laboratory (EMBL) database of gene sequences.

When compared with the SwissProt protein database using the 'Blitz' programme (MPsrch programme, Biocomputing Research Unit, University of 5 Edinburgh, UK) the putative amino acid sequence shows similarity (% 50.9 % similarity, 36.9 % identity) to a cDNA encoding a protein isolated from kiwifruit (Ledger and Gardner, 1994). The steady state level of the kiwifruit transcript increases during fruit development, but declines during ripening. This is in contrast to the expression of the RIB1 gene in blackcurrant fruit where the steady state transcript 10 level increases during the ripening period. Importantly, like the blackcurrant transcript, the kiwifruit gene is expressed almost entirely in the fruit.

pRIB 3

The ORF present in pRIB3 encodes a polypeptide which shares a high degree 15 of sequence similarity with group one metallothioneins. The most similar metallothionein protein to the blackcurrant deduced sequence was from kiwifruit (79% similarity, 67% identity). Typical of metallothioneins, the putative blackcurrant polypeptide has a low M_r value (M_r 6808) and is acidic (pI 4.56). Metallothioneins also contain characteristic cysteine rich domains and the arrangement of these regions 20 in blackcurrant and in a kiwifruit metallothionein is highly conserved. There are two Cys pairs in the N-terminal domain and three Cys pairs in the C-terminal domain separated by a hydrophobic domain. This organisation has also been observed in putative metallothioneins isolated from rice and *Arabidopsis* but differs from some plant sequences where there are three Cys pairs in the N-terminal domain.

25

pRIB 5

A long ORF was also identified in the pRIB5 cDNA sequence, extending from the nucleotide in position 3 to the termination codon in position 777. A methionine initiation codon was not present in this ORF indicating that the cDNA was not full 30 length. Searches of the EMBL database with the deduced amino acid sequence of this ORF and also with the nucleotide sequence did not reveal any significant similarities

to known sequences. The putative amino acid sequence encoded by pRIB5 does not show significant similarity to other amino acid sequences in the SwissProt database.

p RIB 6

5 pRIB6 encodes the C-terminal portion of a polypeptide that shares sequence similarity with the cysteine proteinase family. This group of proteins includes actinidin from kiwifruit, papain from papaya and bromelain from pineapple. The putative protein encoded by pRIB6 shows most similarity to a cysteine proteinase precursor from *Arabidopsis thaliana* (74% similarity, 60% identity), the expression of 10 which is induced by high salt conditions. Five of the highly conserved residues found in or near the active site of all cysteine proteases are present in the blackcurrant sequence.

pRIB7.

15 pRIB7 contains a long ORF extending from a putative methionine initiation codon at nucleotide 29 to a TAA termination codon at position 860. The ORF encodes a protein of M_r 29,215 and a pI of 7.9. However, a common poly(A)⁺ addition sequence is not present. The pRIB7 ORF was most similar to the yeast mitochondrial protein MRS4, a mitochondrial RNA splicing protein (62% similar and 20 42% identical at the amino acid level). Hydropathy plots have shown that the MRS4 protein contains potential membrane spanning domains and analysis of the pRIB7 ORF sequence shows that this may also be the case for the blackcurrant polypeptide. The MRS4 protein contains three repeated amino acid sequences of approximately 100 residues and a characteristic highly conserved domain. Such sequence motifs are 25 also seen in a number of mitochondrial carrier proteins.

RIB 7

The 5150 nucleotide sequence contains a 'TATA box' element at nucleotide 3041 and a putative ATG translational start codon at position 3156. This translational 30 start codon is in the context TTTCAATGGCG and matches the optimal context consensus sequence (NNANNATGGCT), where N is any nucleotide) proposed by Heidecker and Messing (1986) in all but two positions (these are underlined).

By comparison with the cDNA sequence, the RIB 7 gene contains two exons and one intron. The 454 nucleotide intron is located between bases 3927 and 4381. On the basis of the translational start codon being located at position 3156, the putative polypeptide encoded by the RIB 7 gene is composed of 328 amino acids. The 5 deduced amino acid sequence has been compared with others in the SwissProt database and is most similar to a mitochondrial RNA splicing protein (MRS4 : Accession number P32500) from yeast (60.3% similarity and 40.3% identity).

Southern analysis

10 Southern blots of genomic DNA from *R. nigrum* (cvs Ben Alder, Ben Sarek and Baldwin), *R. loganobaccus* (Tayberry) and *R. idaeus* (cv Glen Moy), were hybridised with probes from the RIB genes. Generally, with all these probes, a small number (2 to 4) of hybridising bands were detected by Southern analysis when the genomic DNA was digested with *Bam*HI, *Eco*RI or *Hind*III. This indicates that the 15 RIB genes are present in low copy number in the genomes of these diploid species. Blots probed with RIB3 and RIB5 showed that these or similar sequences are not present in the genomes of raspberry and Tayberry as no hybridising bands could be detected on the Southern blots (data not shown). As a control, these blots were stripped and re-probed with a potato β -tubulin probe which gave multiple 20 hybridisation signals with genomic DNA from all the samples that were probed (data not shown).

Discussion

On the basis of respiration measurements, blackcurrants do not exhibit a typical climacteric pattern of ripening. Additionally, the large increase in ethylene 25 evolution that commonly accompanies the respiratory climacteric was not detected. Compared with the rate of ethylene production from ripening avocado fruit (internal ethylene levels increase 1000-fold between the pre-climacteric and climacteric peak) the amount of ethylene produced by blackcurrant fruit was very low. It is not clear which plant growth regulators trigger ripening processes in blackcurrant fruit.

30 Irrespective of the plant growth regulators that control ripening in blackcurrant fruit, until now, none of the genes that are differentially expressed during fruit ripening have been isolated. A cDNA library constructed from the green/red stage of

ripening was differentially screened with probes from this stage and from green fruit, since genes that are differentially expressed as anthocyanin accumulation commences are good candidates for having an important role in this and other ripening processes. In fact the expression of all five genes corresponding to the isolated cDNAs, 5 continued to increase as ripening progresses and reached a maximum steady-state level in fully ripe, black fruit (Figure 1). The expression of these genes showed varying degrees of fruit specificity. RIB1 and RIB7 were expressed only at very low levels in non-fruit tissues. The promoters driving the expression of these two genes therefore are good candidates for being fruit specific promoters and therefore suitable 10 for use in manipulating ripening processes in transgenic fruit. RIB3, RIB5 and RIB6 were also expressed in roots leaves and stems. RIB3 exhibited a markedly different expression pattern in stems and roots from plants that had not borne fruit (no detectable expression) compared with plants that had (relatively high steady-state transcript levels). It seems likely that the expression of these genes is highly regulated 15 in a tissue- and developmental-stage specific manner.

In order to determine the copy number and occurrence of the RIB genes in other soft fruit species, Southern blot analyses were performed. Of the five clones isolated from the cDNA library, three of them, pRIB1, pRIB6 and pRIB7 hybridised to DNA from three blackcurrant cultivars, Tayberry and red raspberry. These clones, 20 may represent genes that occur widely in soft fruit species. Interestingly, in Southern blots probed with pRIB3 and pRIB5, hybridising bands were only present in lanes containing blackcurrant DNA, suggesting these genes and related sequences are absent in other soft fruit species.

It was possible to identify tentatively three of the blackcurrant sequences, 25 based on similarity searches of databases. Sequences similar to pRIB3, encoding a metallothionein-like protein and pRIB6, encoding a cysteine proteinase have been found previously to be expressed in many plant species. A number of highly conserved amino acid residues, essential for protease activity, are present in the putative blackcurrant sequence.

The pRIB3 ORF has strong sequence similarity to a number of metallothionein-like proteins that have been isolated previously from plants. It is interesting, that of these proteins, the most similar to the pRIB3 sequence, was 30

isolated from the ripening fruit of kiwifruit. Like pRIB3, high steady-state transcript levels of the kiwifruit gene were detected in ripe fruit. In animals, metallothioneins function to maintain metal ion homeostasis and are involved in metal ion detoxification. Additionally they may provide protection against oxidative stress.

5 Although no similar functions have yet been demonstrated for plant metallothioneins, it is possible that they have similar roles. Indeed plant metallothionein-like proteins have been shown to bind cadmium and copper. However it is unclear at the moment, why the steady-state level of the metallothionein-like protein specific transcript increases in ripe fruit. It is interesting that DNA sequences hybridising to the RIB3

10 probe on the Southern blot were only present in blackcurrant, and not in raspberry or Tayberry.

pRIB7 was most significantly similar to a gene that has not been previously found to be expressed in plants, the yeast MRS4 gene. This nuclear gene encodes a mitochondrial RNA splicing protein. Although most similar to the MRS4 gene product, the pRIB7 ORF shares some sequence motifs with a number of mitochondrial carrier proteins such as the phosphate carrier protein and the ADP/ATP translocase. The mitochondrial carrier family is characterised by three tandem repeats of a domain of approximately 100 residues, and a highly conserved region within the repeated domain serves as a signature pattern. This consensus pattern (P-Xaa-[D,E]-

15 Xaa [L, I, V, A, T]-[R, K]-Xaa-[L,R]-[L, I, V, M, F, Y]) is found three times in the pRIB7 ORF although one amino acid residue in the repeat in the -COOH-domain differs from this consensus pattern (Q in place of L or R). The role of the pRIB7 polypeptide therefore is unknown but it may be related to changes in solute transport across the mitochondrial membrane, reflecting changes in metabolism as fruit ripen.

20 The pRIB1 and pRIB5 ORFs did not show any sequence similarity to known sequences in the EMBL database.

25

REFERENCES

Bevan, M. (1984). *Nucl. Acid. Res.* 12, 8177.

30 Callahan, H., Morgens, P and Walton, E (1989). *Hortsci.* 24, 356-358

Fromm, M.E., Taylor, L.P. & Walbot, V. (1985). *Proc. Natl. Acad. Sci. USA* 82, 5824-8.

Heidecker, G. and Messing, J. (1986). *Ann. Review of Plant Physiology*, 37, 439-466.

Klein, T.M., Wolf, E.D., Wu, R. & Sandford, J.C. (1987). *Nature* 327, 70-73.

5 Lay-Yee, M., Dellapenna, D. and Ross, G.S. (1990). *Plant Physiol.* 94, 850-853

Ledger, S.E. and Gardner, R. C. (1994) *Plant Mol. Biol.* 25, 877-886

Martin, C., Prescott, A., Mackay, S., Bartlett, J. & Vrijlandt, E. (1991). *Plant J.* 1, 37-49.

10 Sambrook, J., Fritsch, E.F. & Maniatis, T. (1989). *Molecular Cloning: A Laboratory Manual*, 2nd Edn. Cold Spring Harbor Laboratory Press.

Schuch, W., Kanczler, J., Robertson, D., Hobson, G., Tucker, G., Grierson, D., Bright, S., Bird, C. *Hortsci* 26, 1517-1520. (1991).

Tesniere, C. & Vayda, M.E. (1991). *Plant Mol. Biol. Reptr.* 9, 242-251.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

5

(i) APPLICANT:

- (A) NAME: SmithKline Beecham plc
- (B) STREET: New Horizons Court
- (C) CITY: Brentford
- 10 (D) STATE: Middlesex
- (E) COUNTRY: England
- (F) POSTAL CODE (ZIP): TW8 9EP
- (G) TELEPHONE: 0181 975 6334
- (H) TELEFAX: 0181 975 6177

15

(ii) TITLE OF INVENTION: Novel product and process

(iii) NUMBER OF SEQUENCES: 15

20

(iv) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- (D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

25

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

30

- (A) LENGTH: 882 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: unknown
- (D) TOPOLOGY: unknown

35

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(vi) ORIGINAL SOURCE:

5 (A) ORGANISM: *Ribes nigrum*
 (B) STRAIN: Ben Alder

10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

	CAGCATTCCA AGAGGAAAAA AAACATGATC AAGAAGTAAT TACTACAAA GAGGAAGCTG	60
	TAGTAGTAAC TGCACCACCA CCATCAGAAA CAGCAGAGCC AGCTGCAGCT GTTGTGCCG	120
15	AGGAAGAGAC AACAAAGGAG CAAGAAGAGC CGCCAGCAGT ATCGGCCGAG GAACCTGTGG	180
	CCCCAGCTGA AGTAGAGACA AAGGTGGAAG TTACAGAAGA ACCACCAAAA GTTGAGGAGA	240
20	AACCAGCAGA AGTAGAGGAG GCTCCAAAGG AAACAGTACA AACAGAACCA GCTGTTGAGA	300
	AGACCATCAA GGAGGAAACT GTAGAGGACT CTGTCGTGGC ACCTGCTCCC GAACCGGAAG	360
	CCGAAGTCCC AAAAGAGAAG GTAATTGCTA CTACTGAAAC TACTGAGGAA GAAGAAAAAG	420
25	TGGCAGTTGA AGAAGTTGAA GTGAAAGTTG AAACAGAGGA GGGAGAAGTT ACTGAGGAGA	480
	AGACTGAGTA AAATAAGTTG TACAACATT ATTGCACGC CTTATTTCT CAATTGGAAG	540
30	TTTATAATGT AGTGGGCTTT TGGTAATATT TGGGGGTTA ATAAGTGGTT TAAGTGGTT	600
	AAGGCTTTT TGGAATTAG ATATTTGGGT AAAGGCCTAC TTGAACAAAA CATAGAAATT	660
	TGGCACACAT GGGTAAAAGT CAAACTTGT TGAGGATGTT TTCTTGTGG TTAAATGTGT	720
35	GTGCCAAGTA GTAGAATGTG GTGGTTGTA TGTAAGTTCT CAAGTAGGGT TTATGAGTCC	780
	TAGTATTATG CTTGATTGTA TGTTGATATG AAAATGGGGG TATGTTGGCT TTGAATAAAA	840

GTTTTAATT TTATAAAAAA AAAAAAAA AAAAAAAA AA

882

(2) INFORMATION FOR SEQ ID NO: 2:

5 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 162 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: unknown
- (D) TOPOLOGY: unknown

10

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: YES

15 (iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(vi) ORIGINAL SOURCE:

20 (A) ORGANISM: Ribes nigrum
(B) STRAIN: Ben Alder

25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Ala Phe Gln Glu Glu Lys Lys His Asp Gln Glu Val Ile Thr Thr Lys
1 5 10 15

30 Glu Glu Ala Val Val Val Thr Ala Pro Pro Pro Ser Glu Thr Ala Glu
20 25 30

35 Pro Ala Ala Ala Val Val Ala Glu Glu Glu Thr Thr Lys Glu Gln Glu
35 40 45

35 Glu Pro Pro Ala Val Ser Ala Glu Glu Pro Val Ala Pro Ala Glu Val
50 55 60

Glu Thr Lys Val Glu Val Thr Glu Glu Pro Pro Lys Val Glu Glu Lys

65

70

75

80

Pro Ala Glu Val Glu Glu Ala Pro Lys Glu Thr Val Glu Thr Glu Pro

85

90

95

5

Ala Val Glu Lys Thr Ile Lys Glu Glu Thr Val Glu Asp Ser Val Val

100

105

110

Ala Pro Ala Pro Glu Pro Glu Ala Glu Val Pro Lys Glu Lys Val Ile

10

115

120

125

Ala Thr Thr Glu Thr Thr Glu Glu Glu Glu Lys Val Ala Val Glu Glu

130

135

140

15

Val Glu Val Lys Val Glu Thr Glu Glu Gly Glu Val Thr Glu Glu Lys

145

150

155

160

Thr Glu

20

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 519 base pairs

25

(B) TYPE: nucleic acid

(C) STRANDEDNESS: unknown

(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

30

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

35

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Ribes nigrum

(B) STRAIN: Ben Alder

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

AAACAAACAAA CTTTTTCATC AATCTTCTTT CTTTAATCAT CACCATGTCG AGCTGCGGAA 60
5 ACTGCGACTG TGCCGACAAG ACCAACTGCC CAAAGAAGGG AAACAGCTAC GGCTTGACA 120
TCATTGAGAC CCAGAAGAGC TACGATGACG TCGTGGTGAT GGATGTTCAAG GCAGCTGAGA 180
10 ATGATGGCAA GTGCAAAGTGC GGCCCGAGCT GCAGTTGTGT GGGCTGCAGC TGTGGTCATT 240
AAGTTAAACA CAACATTATC ATGTTATAGT GAATAATGAT GTGTGTGATG AATATAGGTG 300
AAAAATCTGT GGTGTGATAA AAACCGTTGG TGAATAAATA GGTGTATATT TCGTGTGCAC 360
15 CTTCTACGAG TACTTGTGCT TGTTGGGTGA AAGAAATATG CACCTAAGTG TCAGTTGTTT 420
TCCGTGTTTT TCGCCGTGTC CCTTGTAAATG GTCATGTTG TGTTTCTTG TGGTTAAATT 480
20 AAATGAACTA GTAATGTTAT GTAAAAAAAAA AAAAAAAAAA 519

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

25 (A) LENGTH: 65 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

30 (ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

35 (v) FRAGMENT TYPE: N-terminal

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Ribes nigrum*

(B) STRAIN: Ben Alder

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Met Ser Ser Cys Gly Asn Cys Asp Cys Ala Asp Lys Thr Asn Cys Pro
1 5 10 15

10

Lys Lys Gly Asn Ser Tyr Gly Phe Asp Ile Ile Glu Thr Gln Lys Ser
20 25 30

15

Tyr Asp Asp Val Val Val Met Asp Val Gln Ala Ala Glu Asn Asp Gly
35 40 45

20

Lys Cys Lys Cys Gly Pro Ser Cys Ser Cys Val Gly Cys Ser Cys Gly
50 55 60

His

25

65

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

25

- (A) LENGTH: 1046 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: unknown
- (D) TOPOLOGY: unknown

30

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

35

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Ribes nigrum
- (B) STRAIN: Ben Alder

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

5	GGAGGAGATC ACCAGTTCCA CCAACACGTC GTCGTAATGA GACACGGCGA TCGGATAGAC	60
	AACTTCGAGC CACTGTGGGT GAAGACGGCG GCGAACGATG GGACCCACCC TTGGTCGATG	120
	AAGGCAAGCT CCGTACCTTC CGGACAGGTC TGAAGCTCCG AACCAATTG GATTTCCGA	180
10	TCCATCGTGT CTTTGTATCA CCTTTCCCTCC GGTGCGTACA GACAGCATCG GAAGTCATCT	240
	CCGCTCTCTG CGCCGTCGAC GATATTCCCG CCACCACTAA TAGAGGCGAT CAAGTACAAA	300
15	TCGATCCATC CAAGATCAAG GTCTCTATTG AGTATGGATT ATGTGAAATG TTGAACATGC	360
	AAGCCATAAG ACTTGGTATG GATTTCAGCA ATGGGAATTG GGGTTTCGAT AAATCACACC	420
	TTGAATCAAC ATTCCCAGTT GGGACGGTGG ATCATAGTGT GGAACCACTC TATAAAGAGA	480
20	TGCCAAAATG GGAAGAGACA GTCAATGGCG CAAGGGCCAG ATATGAAGAG GTTATTCAAGG	540
	CCCTAGCAGA TAAATACCCC ACGGAGAACT TGTTGCTTGT TACACATGGG GAAGGAGTTG	600
25	GCGTTGCAGT TTCTGCCTTC ATGAAGGATG TTACAGTGT ACGAAGCCGAT TATTGTGCCT	660
	ATACACACGC AAGAAGATCC ATTGTCTTGG GCAAAAACCA GTCATTTACT GCTGAAAAC	720
	TTGAAGTATT ACCAAAACAA GGCCAAACTG GTGTCAGTTA CGTCCTTGAA CAGCATTGAT	780
30	GGAACTGTAT GACCTAATTG TGGCAGCCGA TGATTACAGA AACAAATTCC ACACCTTTT	840
	TCTTTTTTCG GGCATTTGCC TACATTTAT AATTAATTAG GCATTCTCAT AGCTAAGGCT	900
35	CATTGGATTC ACATCCCTAC TTGTTAAAG GAGACTTGA TTTGTTGCCT CCAAACAGAA	960
	CATATGTTGC TGTGTCCATC AGCTTTTTT AACTGGGATT TCTATTTTA CAGTGTGTAA	1020
	AAAAAAAAAAA AAAAAAAAAA AAAAAAA	1046

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

5 (A) LENGTH: 258 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

10 (ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: YES

15 (iv) ANTI-SENSE: NO

15 (v) FRAGMENT TYPE: N-terminal

20 (vi) ORIGINAL SOURCE:

20 (A) ORGANISM: Ribes nigrum
(B) STRAIN: Ben Alder

25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

Arg Arg Ser Pro Val Pro Pro Thr Arg Arg Arg Asn Glu Thr Arg Arg
1 5 10 15

30 Ser Asp Arg Gln Leu Arg Ala Thr Val Gly Glu Asp Gly Gly Glu Arg
20 25 30

Trp Asp Pro Pro Leu Val Asp Glu Gly Lys Leu Arg Thr Phe Arg Thr
35 40 45

35 Gly Leu Lys Leu Arg Thr Asn Phe Asp Phe Pro Ile His Arg Val Phe
50 55 60

Val Ser Pro Phe Leu Arg Cys Val Gln Thr Ala Ser Glu Val Ile Ser
65 70 75 80

Ala Leu Cys Ala Val Asp Asp Ile Pro Ala Thr Thr Asn Arg Gly Asp
85 90 95

5 Gln Val Gln Ile Asp Pro Ser Lys Ile Lys Val Ser Ile Glu Tyr Gly
100 105 110

Leu Cys Glu Met Leu Asn Met Gln Ala Ile Arg Leu Gly Met Asp Phe
115 120 125

10 Ser Asn Gly Asn Trp Gly Phe Asp Lys Ser His Leu Glu Ser Thr Phe
130 135 140

Pro Val Gly Thr Val Asp His Ser Val Glu Pro Leu Tyr Lys Glu Met
15 145 150 155 160

Pro Lys Trp Glu Glu Thr Val Asn Gly Ala Arg Ala Arg Tyr Glu Glu
165 170 175

20 Val Ile Gln Ala Leu Ala Asp Lys Tyr Pro Thr Glu Asn Leu Leu Leu
180 185 190

Val Thr His Gly Glu Gly Val Gly Val Ala Val Ser Ala Phe Met Lys
195 200 205

25 Asp Val Thr Val Tyr Glu Ala Asp Tyr Cys Ala Tyr Thr His Ala Arg
210 215 220

Arg Ser Ile Val Leu Gly Lys Asn Gln Ser Phe Thr Ala Glu Asn Phe
30 225 230 235 240

Glu Val Leu Pro Lys Gln Gly Gln Thr Gly Val Ser Tyr Val Leu Glu
245 250 255

35 Gln His

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1017 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: unknown
- 5 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

10

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Ribes nigrum
- 15 (B) STRAIN: Ben Alder

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

20

GTTGATGGCA GATGTGACCA ACTCAGGAAA AATGCCAGGG TTGTTGCAAT TGATTCTTAC 60

GAAGATGTTCTTTAACGATGAGAACGCA TTGAAAAAAGG CAGTGGCTAG TCAGCCTGTG 120

25

CGCGTCGCCA TTGAAGGAGG TGGCAGGGAT TTCCAATCT ATCAATCAGG CGTCTTTACT 180

GGATCATGTG GGACGGCCCT AGACCATGGT GTGGCTGCTG TTGGGTATGG CACAGAAAAT 240

30

GGTGTGGATT ACTGGATTGT AAGGAACTCA TGGGGTGCAA GCTGGGGAGA GAGCGGCTAC 300

ATCAGGATGG AACGTAATCT GGCAGGCACA GCTACGGGCA AATGTGGTAT TGCAATGGAA 360

GCCTCTTACC CTATTAAGAA AGGCCAAAT CCCCCAAACC CAGGACCATC TCCTCCATCT 420

35

CCAATAAAGA CCTCCAACAG TTTTGACATTACATTATAC CTTGGCTGAA AGCACCACCT 480

GCTGCTGTCT ATTTGAGTTT GGCAGGTATT GCTTCGAGTG GGGATGTTGC CCACTCGAGG 540

CTGCCACTTG CTGTGATGAC CATTACAGTT GCTGCCACCA TGAGTATCCC ATCTGCAACC 600

TTAATGCAGG GACGTGTATG ATGAGAAGGA CAACCCATTG AGTGTGAAGG CATTGAAGCG 660

5 TACTCCGCT AAACCTCATT GGGCCTTGG GAACCGTGGC AAGAGCAGCA GTGCTTAAGA 720

ACATTGTGTC ATCTATACAG TGAAAGTAAA ACGAGGATGA AAAGTTGTAT CAGGCAGGGC 780

TTGATGATCT CCTCGGTTT ATAGTACCGC ATACCCTCAT TCTCCATTAA GGTCATATAC 840

10 ATATGGACGG TTTATCAAAG TTTATTCAAGA TGCTAATTAT GTATATATCA TTTCTCAGTC 900

TCTGTATTTC ATTTAACGA GAACATAAAC AGATCGTTAT CAGCTACCAA TTTCCACTGT 960

15 AAATCACGTT ATCAATTATT TACTGGCCTC GCTGAAAAAA AAAAAAAA AAAAAAA 1017

(2) INFORMATION FOR SEQ ID NO: 8:

20 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 206 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: unknown

(D) TOPOLOGY: unknown

25 (ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: YES

30 (iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

35 (vi) ORIGINAL SOURCE:

(A) ORGANISM: Ribes nigrum

(B) STRAIN: Ben Alder

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

WO 97/17452

PCT/EP96/04807

Val Asp Gly Arg Cys Asp Gln Leu Arg Lys Asn Ala Arg Val Val Ala
1 5 10 15

Ile Asp Ser Tyr Glu Asp Val Pro Leu Asn Asp Glu Asn Ala Leu Lys
5 20 25 30

Lys Ala Val Ala Ser Gln Pro Val Arg Val Ala Ile Glu Gly Gly
35 40 45

10 Arg Asp Phe Gln Leu Tyr Gln Ser Gly Val Phe Thr Gly Ser Cys Gly
50 55 60

Thr Ala Leu Asp His Gly Val Ala Ala Val Gly Tyr Gly Thr Glu Asn
65 70 75 80

15 Gly Val Asp Tyr Trp Ile Val Arg Asn Ser Trp Gly Ala Ser Trp Gly
85 90 95

Glu Ser Gly Tyr Ile Arg Met Glu Arg Asn Leu Ala Gly Thr Ala Thr
20 100 105 110

Gly Lys Cys Gly Ile Ala Met Glu Ala Ser Tyr Pro Ile Lys Lys Gly
115 120 125

25 Gln Asn Pro Pro Asn Pro Gly Pro Ser Pro Pro Ser Pro Ile Lys Thr
130 135 140

Ser Asn Ser Phe Val Thr Ile Thr Ile Pro Trp Leu Lys Ala Pro Leu
145 150 155 160

30 Ala Ala Val Tyr Leu Ser Leu Ala Gly Ile Ala Ser Ser Gly Asp Val
165 170 175

Ala His Ser Arg Leu Pro Leu Ala Val Met Thr Ile Thr Val Ala Ala
35 180 185 190

His Met Ser Ile Pro Ser Ala Thr Leu Met Gln Gly Arg Val
195 200 205

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

5 (A) LENGTH: 1311 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

10

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

15

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Ribes nigrum
(B) STRAIN: Ben Alder

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

GACGCCACTC ACCCTGAATT TCTCCACGTA CCAAAACCTA AACCTCATGA ATTCCACCCA 60
25 GAAATCTCTA TCGCGCCGTC GCATGATGGC CTTCAGTTCT GGCAGTTCAT GATCGCCGGT 120
TCAATCGCTG GATCAATCGA GCATATGGCG ATGTATCCGG TTGATAACGCT TAAAACTCGC 180
30 ATACAGGCTA TTGGGTCTAG TTCCGGCTCAA TCCGCCGGTC TCCGACAAGC CCTTGGGTCTG 240
ATACTGAAAG TTGAAGGTCC CGCCGGACTT TACCGTGGCA TTGGTGAAT GGGTCTCGGT 300
GCAGGACCAG CTCACGGCAGT GTATTTCTCC GTTTACGAGA TGTGTAAGGA GACTTTTCT 360
35 CATGGTGATC CGAGCAATTG CGGTGCGCAC GCCGTTCTGG GGGTGTTCGC GACGGTGGCA 420
AGCGACGGCGG TGATTACGCC GATGGATGTG GTGAAACAGA GGTTGCAGTT GCAGAGCAGT 480
CCGTACAAGG GTGTTGTTGA TTGCGTGAGG AGGGTGTGG TAGAAGAAGG GATTGGCGCA 540

TTTTACGCAT CTTATCGAAC AACTGTGGTC ATGAATGCC CGTTTACGGC CGTTCACCTC 600
GCCACATATG AAGCCACGAA GAAAGGGTTG TTGGAGGTGT CGCCGGAGAC TGCGAACGAT
5 GAGAATTGT TAGTGCATGC TACTGCTGGT GCTGCTGCTG GAGCTTGGC TGCAGTAGTA
ACCACTCCAC TAGATGTTGT CAAAACTCAG TTGCAGTGCC AAGGTGTTG CGGATGCGAC 780
10 AGATTTCTA GCAGTTCGAT TCAGGATGTT ATAGGAAGCA TAGTGAAGAA AAATGGATAT
GTCGGGTTAA TGAGGGGGTG GATTCCCAGA ATGCTATTTC ATGCTCCTGC TGCAGCAATC
TGCTGGTCTA CTTATGAAGC CTCCAAAACA TTCTTCAAA AACTCAATGA GAGCAATAGC 960
15 AACAGCTCAG TTACCTAAGA TTTCATATGT TTTGTTGCT CTACTAGGCT TATCCAAAAT
CATGTCGATT GGTTTCACTT CACCACAGTT GCCATGAACA ACTCAAAGCA TCGAATTTA
20 CATGTATATT ATGCAATCTA GATGCTCTT GATATTATT TTTATTTTT CTTTTCCAAC
TTTGTAATT AGAATTAGCT ACTATGGTTA TGGCATGGAG TGTTTATAA TTGCTAATAT
CATCGTATAA GCAATGCTAT TTGAGAAATT GTGGTGTAAAG GTTAGAGTAA TGTTATTTGC 1260
25 ACAATCCACT TACATAGACC GCGGGACTCA TTTAAAAAAA AAAAAAAA A 1311

(2) INFORMATION FOR SEQ ID NO: 10:

30 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 289 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: unknown
- (D) TOPOLOGY: unknown

35

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

5 (vi) ORIGINAL SOURCE:

(A) ORGANISM: Ribes nigrum

(B) STRAIN: Ben Alder

10

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

Met Ile Ala Gly Ser Ile Ala Gly Ser Ile Glu His Met Ala Met Tyr
1 5 10 15

15

Pro Val Asp Thr Leu Lys Thr Arg Ile Gln Ala Ile Gly Ser Cys Ser
20 25 30

20

Ala Gln Ser Ala Gly Leu Arg Gln Ala Leu Gly Ser Ile Leu Lys Val
35 40 45Glu Gly Pro Ala Gly Leu Tyr Arg Gly Ile Gly Ala Met Gly Leu Gly
50 55 60

25

Ala Gly Pro Ala His Ala Val Tyr Phe Ser Val Tyr Glu Met Cys Lys
65 70 75 80

30

Glu Thr Phe Ser His Gly Asp Pro Ser Asn Ser Gly Ala His Ala Val
85 90 95Ser Gly Val Phe Ala Thr Val Ala Ser Asp Ala Val Ile Thr Pro Met
100 105 110

35

Asp Val Val Lys Gln Arg Leu Gln Leu Gln Ser Ser Pro Tyr Lys Gly
115 120 125Val Val Asp Cys Val Arg Arg Val Leu Val Glu Glu Gly Ile Gly Ala
130 135 140

WO 97/17452

PCT/EP96/04807

Phe Tyr Ala Ser Tyr Arg Thr Thr Val Val Met Asn Ala Pro Phe Thr
145 150 155 160

Ala Val His Phe Ala Thr Tyr Glu Ala Thr Lys Lys Gly Leu Leu Glu
5 165 170 175

Val Ser Pro Glu Thr Ala Asn Asp Glu Asn Leu Leu Val His Ala Thr
180 185 190

10 Ala Gly Ala Ala Ala Gly Ala Leu Ala Ala Val Val Thr Thr Pro Leu
195 200 205

Asp Val Val Lys Thr Gln Leu Gln Cys Gln Gly Val Cys Gly Cys Asp
210 215 220

15 Arg Phe Ser Ser Ser Ser Ile Gln Asp Val Ile Gly Ser Ile Val Lys
225 230 235 240

Lys Asn Gly Tyr Val Gly Leu Met Arg Gly Trp Ile Pro Arg Met Leu
20 245 250 255

Phe His Ala Pro Ala Ala Ile Cys Trp Ser Thr Tyr Glu Ala Ser
260 265 270

25 Lys Thr Phe Phe Gln Lys Leu Asn Glu Ser Asn Ser Asn Ser Val
275 280 285

Thr

30

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1797 base pairs
35 (B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

5

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Ribes nigrum

(B) STRAIN: Ben Alder

10

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

	GATCTTATAT TGAGGATGCA AAGTTCAAA TTACCTGATA TGTAACCTCTC AACAAAATCA	60
15	AGCTTTGAT CATATAAAC GAAACCAACA CACAATAATT ATGAATTCT TTGACTCTTT	120
	GTCTCTGTAC CAAAATACGC ACACCACAAA AAATTCTTT TGTATTATAT TCGTTTTTA	180
20	TTTTTTAAC GTTTGGTAT TCAAAACATCA TATAAGTAAG GGGGAATATT ATTGGACTC	240
	CTCCAAAAAC TTATGACATT GTGATTACAC ATTTGAATGA CAGAAGTTT TGATGAAGTG	300
	CCAATATCAA TCTTTCTTA ATTGCTTCAT AAAGGGTGT TTTGTAATTA AAAGAAAGAT	360
25	AAGGAAATT AGCAAGAAGT GCATTATTGG GACTGGTATA TATGACAAGG ATCTGACGTG	420
	GCAAAGAAAG AAAGTGGGTC CTGAGTCAGG TGTGTCCCAT CTGTCAATAT TCTTCAAAAG	480
30	AGAGTCCACC ATCTCATAGA TGAGATTTAG AAAGTGGTTT CCACAAAAAA ATATGACACA	540
	ACCCATCCAT GAACCAATAA AAACATGACA GGTCATCATT TCTTTCTATT TTTTCTCTC	600
	AAGATAATAA TACCTATTAG TGTCTTTAAC ACCGGCCTAA CTTGCATTT CTTGTCATTT	660
35	GGTGACTTT TATTGCCAA TTGTGGCTTG AAGGAAATAA AAAGGAAAGT CTTTTCTTG	720
	AACCCATATG GAAGCAATT CAATGAGAGA GATAGAGAGG AGGGATGGAG ATTGGGGTGG	780

	AGAATTGATA CGGATCTTCT TTAATTGGTA TATGTAAATC ACTCAGAAAC ACGTATAACCA	840
	TATATGCATC AATGTCAATG TCACAGAAAA CGTAACTCAC GAACACATTT CGTAACATGC	900
5	ATGCACCAAT CATACTATT AACATAGTGT TACGACAATA AAAGATCTTT AGTCGTAAGA	960
	GCATTAGCTC GTGACAAGAA CAAAAACGTG GATTCCCAAC CTAAAGAAGG GTATATCTTT	1020
	TATTCATATA TCTACTTTG ATATGACCTA AACCTTGTGT CACCCACAAT GTTCAGTACG	1080
10	ATCGATAATT GTTGACTTG TGTGGGATGA GAAAATGTAT GAGACTGGCC ATTAGTTTA	1140
	GCCGGATGTG ATTTGGGTAT ATTGATGACA ATATAAGATA TATAAAACTT GAACAAAACA	1200
15	ATTTCTCAAC AAATTAAACT ACAAGATAAT CTCCCTTCAG ATGATAAACT AAATGGTAGA	1260
	ATATCCGTTG AGTACCCCCA ATAATTAAA ATCTCCAGCA AATACTGTGA TTCCTTTCT	1320
	TCGAAGCGAA ATTCTTCCT TCCAAACACC TTAACAAATG TAAAATTGTT TAGTAAGATT	1380
20	AAATTGAAA TGATAACACA AGAGTGAATA AAGGTCACTGG TCACCTACTT ACCCAACTGC	1440
	ACAAAACACA CAAGCACACA TCCAAAAGTA GTAGTATGAT TACACACATT TGAAAAAATG	1500
25	ACCTCCATTA TTTAGCCAC CTCTCTGTAA AAAAGATTA CAAACAAATT ACTCCTATCA	1560
	TTATTATAAA AATAGTAGCA TAACCTCATC TCCAATCCAC ACCATATATT TTACATTATT	1620
	GCCAAACATG CTAAAAGCTT CTTGTATTCA GTGAAAATGT GGTGTCAAAT CCCAAGATTC	1680
30	TTCATGTGCC CTCTCTCTCT CTCTCTCTCT CTCTCCTCCT CCTCCTCCTC TCTCTCTCTC	1740
	ATCAAATTGA GGGCTTTAGG ACCTCTATAT AAACCTCTCT CAATTGATCA TCTCTGC	1797
35	(2) INFORMATION FOR SEQ ID NO: 12:	

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 3292 base pairs
- (B) TYPE: nucleic acid

(C) STRANDEDNESS: unknown
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

5

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

10 (vi) ORIGINAL SOURCE:

(A) ORGANISM: *Ribes nigrum*
 (B) STRAIN: Ben Alder

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

GATCTTATAT	TGAGGATGCA	AAGTTCAAA	TTACCTGATA	TGTAACCTCTC	AACAAAATCA	60	
20	AGCTTTGAT	CATATAAAC	GAAACCAACA	CACAATAATT	ATGAATTCT	TTGACTCTTT	120
	GTCTCTGTAC	CAAAATACGC	ACACCACAAA	AAATTCTTT	TGTATTATAT	TCGTTTTTA	180
25	TTTTTTAAC	GTGGTAT	TCAAACATCA	TATAAGTAAG	GGGAATATT	ATTCGGACTC	240
	CTCCAAAAAC	TTATGACATT	GTGATTACAC	ATTTGAATGA	CAGAAGTTT	TGATGAAGTG	300
	CCAATATCAA	TCTTTCTTA	ATTGCTTCAT	AAAGGGTGT	TTTGTAAATT	AAAGAAAGAT	360
30	AAGGAAATT	AGCAAGAAGT	GCATTATTGG	GAUTGGTATA	TATGACAAGG	ATCTGACGTG	420
	GCAAAGAAAG	AAAGTGGGTC	CTGAGTCAGG	TGTGTCCCCT	CTGTCAATAT	TCTTCAAAAG	480
35	AGAGTCCACC	ATCTCATAGA	TGAGATTAG	AAAGTGGTTT	CCACAAAAAA	ATATGACACA	540
	ACCCATCCAT	GAACCAATAA	AAACATGACA	GGTCATCATT	TCTTCTATT	TTTTCTCTC	600
	AAGATAATAA	TACCTATTAG	TGTCTTTAAC	ACCGGCCTAA	CTTGCATTT	CTTGTCAATT	660

WO 97/17452

PCT/EP96/04807

	GGTGACTTTT TATTGCCCAA TTGTGGCTTG AAGGAAATAA AAAGGAAAGT CTTTTCTTG	720
	AACCCATATG GAAGCAATTT CAATGAGAGA GATAGAGAGG AGGGATGGAG ATTGGGGTGG	780
5	AGAATTGATA CGGATCTTCT TTAATTGGTA TATGTAAATC ACTCAGAAAC ACGTATAACCA	840
	TATATGCATC AATGTCAATG TCACAGAAAA CGTAACTCAC GAACACATTT CGTAACATGC	900
	ATGCACCAAT CATACTATT AACATAGTGT TACGACAATA AAAGATCTT AGTCGTAAGA	960
10	GCATTAGCTC GTGACAAGAA CAAAAACGTG GATTCCCAAC CTAAAGAAGG GTATATCTT	1020
	TATTCATATA TCTACTTTG ATATGACCTA AACCTTGTGT CACCCACAAT GTTCAGTACG	1080
15	ATCGATAATT GTTTGACTTG TGTGGGATGA GAAAATGTAT GAGACTGGCC ATTGTTTTA	1140
	GCCGGATGTG ATTTGGGTAT ATTGATGACA ATATAAGATA TATAAAACTT GAACAAAACA	1200
	ATTTCTCAAC AAATTAAACT ACAAGATAAT CTCCCTTCAG ATGATAAACT AAATGGTAGA	1260
20	ATATCCGTTG AGTACCCCCA ATAATTTAAA ATCTCCAGCA AATACTGTGA TTCCTTTCT	1320
	TCGAAGCGAA ATTCCCTCCT TCCAAACACC TTAACAAATG TAAAATTCGT TAGTAAGATT	1380
25	AAATTTGAAA TGATAACACA AGAGTGAATA AAGGTCACTGG TCACCTACTT ACCCAACTGC	1440
	ACAAAACACA CAAGCACACA TCCAAAAGTA GTAGTATGAT TACACACATT TGAAAAAATG	1500
	ACCTCCATTA TTTTAGCCAC CTCTCTGTG AAAAAGATTA CAAACAAATT ACTCCTATCA	1560
30	TTATTATAAA AATAGTAGCA TAACCTCATC TCCAATCCAC ACCATATATT TTACATTATT	1620
	GCCAAACATG CTAAAAGCTT CTTGTATTCA GTGAAAATGT GGTGTCAAAT CCCAAGATTC	1680
35	TTCATGTGCC CTCTCTCTCT CTCTCTCTCT CTCTCCTCCT CCTCCTCCTC TCTCTCTC	1740
	ATCAAACTTGA GGGCTTTAGG ACCTCTATAT AAACCTCTCT CAATTGATCA TCTCTGCATC	1800
	ACACTCTCAA GCATTCTTC TCTCTACTTT CTTTTAGGTC AACTACACTT CCCTTTGAGT	1860

	TTCCAATGGC CACTGTTGAG GTAAATCAAG TGATATATAC ATAAATTTA TTTGAAAGAT	1920
	GATTGATTCA AAGAGAACCC TTTTGTGTTT TCTTTAATAA GATCCATGTA TATGAAGTTT	1980
5	TAATGTTCA TGTTTTTTA TTTTTGTTA ATTTTTTTT AATTAGGCA TTTTGCAAT	2040
	ATCCCATTG TGAAAAGATC TGTTTCCTT TGGAAGAGAT TAGAATTGCT TTCGTGTCGA	2100
10	TTCATCATGA AAATCAATCT GGGTCTAGCT TTAATTGTGC TGATCTTGAC CGGACTGTTA	2160
	GATGATTGCT TTTATATGTA GGCCCAATAG AGAGTGATAG TATTCCGAA ATAATACAAA	2220
	TCCGAGCAAA CTATAATCCT CAATAGTAAC TTTGTAATCT CTAAATAATC AAAAAATAAT	2280
15	GCTTATTGGG GTGATTGGTG TGTTGATGC AGGTTGTATC AGCGCAGACA GCATTCCAAG	2340
	AGGAAAAAAA ACATGATCAA GAAGTAATTA CTACAAAAGA GGAAGCTGTA GTAGTAAC TG	2400
20	CACCACCACC ATCAGAAACA GCAGAGCCAG CTGCAGCTGT TGTTGCCGAG GAAGAGACAA	2460
	CAAAGGAGCA AGAAGAGCCG CCAGCAGTAT CGGCCGAGGA ACCTGTGGCC CCAGCTGAAG	2520
	TAGAGACAAA GGTGGAAGTT ACAGAAGAAC CACCAAAAGT TGAGGAGAAA CCAGCAGAAG	2580
25	TAGAGGAGGC TCCAAAGGAA ACAGTAGAAA CAGAACCCAGC TGTTGAGAAG ACCATCAAGG	2640
	AGGAAACTGT AGAGGACTCT GTCGTGGCAC CTGCTCCGA ACCGGAAGCC GAAGTCCCAA	2700
30	AAGAGAAGGT AATTGCTACT ACTGAAACTA CTGAGGAAGA AGAAAAAGTG GCAGTTGAAG	2760
	AAGTTGAAGT GAAAGTTGAA ACAGAGGAGG GAGAAGTTAC TGAGGAGAAG ACTGAGTAAA	2820
	ATAAGTTGTA CAACTATTT ATGCACGCCT TATTTCTCA ATTGGAAAGTT TATAATGTAG	2880
35	TGGGCTTTG GTAATATTTG GGGGTTTAAT AAGTGGTTA AGTGGGTTAA GGCTTTTTG	2940
	GAATTAGAT ATTTGGGTAA AGGCCTACTT GAACAAAACA TAGAAATTG GCACACATGG	3000

GTAAAAGTCA AACTTTGTTG AGGATGTTTT CTTGTTGGTT AAATGTGTGT GCCAAGTAGT 3060

AGAATGTGGT GGTTGTAATG TAAGTTCTCA AGTAGGGTTT ATGAGTCCTA GTATTATGCT 3120

5 TGATTGTATG TTGATATGAA AATGGGGTA TGTTGGCTTT GAATAAAAGT TTTTAATT 3180

ATATAATAAG TGTATTTTG TTTAATATCA TTCTTCATT CTCTCGGATC AACTACTGAT 3240

CATCGCCTTG GTAAGCTATT GCCTCACCAA CTAGCTAATC GAACGCGAGC CC 3292

10

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:

15 (A) LENGTH: 173 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

20 (ii) MOLECULE TYPE: peptide

25 (iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

25 (v) FRAGMENT TYPE: N-terminal

(vi) ORIGINAL SOURCE:

30 (A) ORGANISM: Ribes nigrum
(B) STRAIN: Ben Alder

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

35 Met Ala Thr Val Glu Val Val Ser Ala Gln Thr Ala Phe Gln Glu Glu
1 5 10 15

Lys Lys His Asp Gln Glu Val Ile Thr Thr Lys Glu Glu Ala Val Val

20 25 30

Val Thr Ala Pro Pro Pro Ser Glu Thr Ala Glu Pro Ala Ala Ala Val
35 40 45

5 Val Ala Glu Glu Glu Thr Thr Lys Glu Gln Glu Glu Pro Pro Ala Val
50 55 60

Ser Ala Glu Glu Pro Val Ala Pro Ala Glu Val Glu Thr Lys Val Glu
65 70 75 80

10 Val Thr Glu Glu Pro Pro Lys Val Glu Glu Lys Pro Ala Glu Val Glu
85 90 95

Glu Ala Pro Lys Glu Thr Val Glu Thr Glu Pro Ala Val Glu Lys Thr
15 100 105 110

Ile Lys Glu Glu Thr Val Glu Asp Ser Val Val Ala Pro Ala Pro Glu
115 120 125

20 Pro Glu Ala Glu Val Pro Lys Glu Lys Val Ile Ala Thr Thr Glu Thr
130 135 140

Thr Glu Glu Glu Glu Lys Val Ala Val Glu Val Glu Val Lys Val
145 150 155 160

25 Glu Thr Glu Glu Gly Glu Val Thr Glu Glu Lys Thr Glu
165 170

(2) INFORMATION FOR SEQ ID NO: 14:

30

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5150 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: unknown

35

- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

5 (A) ORGANISM: *Ribes nigrum*
(B) STRAIN: Ben Alder

10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

	AGCTTATGAT TACAACTATA AAATCAATGC GTGGAAATCA CAAAAACTGG AAATGCTATG	60
15	CTATGGACGA TCAACTGATA AAACTGGAAA TAGGACTAAG AACTGTGAGA ACTAAACTAG	120
	AGAAAACCTTA ATGATCTAAA CTAAAAGTGA CAGCATTG GCAAATCTAA AAAGAGAGGT	180
	TCATTGTCTG ATGATTGGTC CTTTCGTGCT TCCTCCTCCT TTGATTTTA TAGGGCTTTC	240
20	ATCATTAAAT ATTACGATTG CCCAGCTGTC CATGATCCGG CCATAAATAG CCGGATATTG	300
	TTGATTGGTA ATGGCTGTGC TTGATTGGCG GTATTTAACCA CCTGCCGTTT TATTTGTAAA	360
	AACCGTTATG GATTCTCTGA TGAGCATAAA CCACGCTGAA TCGGCCTATT GGTGATTGG	420
25	TGTAAGGCCA TACTCTGAAC AGCCTTGGGG ATTCTGATGA CCGTAGATTG GGCCTTAATG	480
	GGCATTATGA TCGTTACTTC GTCTCATGGT AACTCCATT CGCAGTTTA CCTATGGTGT	540
30	TCCTTGTCAAT GAGTGTACCG GTCATTCCA CTTCGTCAGA CACCTTATC AGCCTAATCC	600
	TAGGTCCATT AAAGTCTGGG GACCTGGATT TGTTATCCTC TAAATTAGAA AGACTATCCT	660
	GATCATTTTT GTTCTTCGGT CATTAGCACC TAGGAGGTTT GGCCAGAAC AGTCTCGTCC	720
35	TTTGATCTT TCGGCCTCGC CAGGCCGGT GGGTTCTG ATACAGAACT CGGCCTATAA	780
	GCCGATTAT ATGAGATGTA AACAGACACA AGATTGGTAA GTTATTTCC ATGTCTAAGT	840

WO 97/17452

PCT/EP96/04807

TCGACTCTCC GTGACCGTGA CCGTGACCGT TCTCCCTTG CCCCAAATTG TTAGTTAAC 900

AAAAATACTG GACAATTCT CACTTGAGTA GTTATTCCC ATTTGTTT CAAACTCTAT 960

5 CTGATGCAGC GGATTATGAA AGGTTAAGAA TTAAACAAGA ATATCACGTA TTCTCGTAAG 1020

AAGAAGAAGA ACACAGAGAA AAGTTCTCAG TTTTATTGA TAAAATATGA ATAATAATCC 1080

10 CTAAAACAAC TTAGAAGTCT TGTTAAATA GAAGCTAGCA AATCCTAATA TGAATAGGAA 1140

ACCCTAATAC GAAAATAAGA AATTACGATA AAAACTCAAC AGATAACGAA ATTACGAAAC 1200

TGTCTGAAAA CACTAAAAT TAAATACAAG GTCCTTAATG ACGGAATTG ACTAAAATCA 1260

15 CGAGACCATG TTACTTTGT AACATGTCTT GAAGATCTCG ACGTTCGCA CCAAGTCACC 1320

AAATTCAAC TAATTCAAC ACTATTGCTA CTATTACGA ACCCAAAATT CTCGCAAACA 1380

ACAGATTAA CTTTACAGTC CAAGCTCCCT ACATCAGGCT CCCCTCTTG AAAAGAACTC 1440

20 ATCCTCGATT TTCTTCGAA AATTGAATTG TGCCTCCCA TTGAAATAAA TACTTTGAAT 1500

ATACATTTG CTTCAACCTT TTGGGCTCAA CAAAATCAA CTTTCTTCC ATCTCCAAT 1560

25 TTTGCACAAT ATCCAATAAT AAAGGATTAG AGAGAAAATT TTCAACCCCA ATAAAATCAA 1620

TTTGTGGAT CTCATTAAT TGAATGAAAT CATGATTTT TTGCTCAACA ATTTCTGATT 1680

TTATTGCTT GATTCTTCA TGCAACTCTT CTTGAGAACT ATCTTGCCTA ATAAAATCGC 1740

30 ATGTTTCAT AGACTCAATG GAATCAAAAG TTTCTCCTT CACTTCATTC AAATCATAAA 1800

CATATTCTTC AACTAAATCA ACATCTTGAT TTGATATGAT TTCTTCTACA ACTCCACCTT 1860

35 TATTTGGTT GTCTTCGTTG ATCCCTTGGA TTTCACACAA AGTTGGTTCA TGGTCAACAA 1920

CATGTGCTCT CCACGAAATT CCATCACATG ATTGTTAATA TTTGTTCTT TCACACTATA 1980

TTTATTTCT AATATTGTT CATAATTCCA CGGTAAAAAT TTACTTCCA TGAGTTCCCT 2040

	CATTCTTGAC CAACAACGAA TACGACGTTT ACCTTGATGT TCTCTTGATT CTTGTAATTT	2100
	TAACCACCAAC CATAACGCTG GACCTGCAAG TTTGCGTAAC ACATACCCCC ACTTCTCTTC	2160
5	TTCCGGAATA TTCATATGCT CAAAGAAATC TTCCATGTCC AATACCCAAT CAAGAAAATC	2220
	TTCAAAGTAA ACACAACCGT TGAAACTAGG CATATTATTA TAATACCTAA AATCTCGACG	2280
10	AAGAGAAACA TAAACGTCAA CAAATCGATT AGCCGCTTGA ATCTCTTGAC GAAACTCCTG	2340
	CCGGAGTTCC ATAAAACTCTC CCACAGTCAC CACACTTCCC TCACGTTCAC CGTCCATGAG	2400
	GATGGCTTG ATACCAACTT GACGCAGCGG ATTATGAAAG GTTAAGAATT AAACAAGAAT	2460
15	AGCACGTATT CTCGTAAGAA GAAGAAGAAC ACGGAGAAAA GTTCTCAGTT TTTATTGATA	2520
	AAATATGAAT AATAATCCCT GAAACAACCT AGAAGTCTTG TTTAAATAGA AGCTAGCAA	2580
20	TCCTAATATG AATAGGAAAT CCTAATACGA AAATAAGAAA TTACGATAAA AACTCAACAA	2640
	ATAACGAAAT TACGAAATTG TCTGAAAACA CTAAAACCTA AATACGAGGT CCTTAACGAC	2700
	GGAATTGAC TAAAATCACG AGACCATGTT ATGTAACATG TCTTGAAGAT CTCGACGTT	2760
25	CGCACCAAGT CAACAAATTT CAACATAATT CCAACTGT TACTACTATT CACGAACCCA	2820
	AATTCTCGCA AACAAACCGAT TTAACCTTAC CGTCCAAGCT CCATACATCA CTATCCAACA	2880
30	CAAAAATGAA AGAACATACA ATTTTACAAA CTTCATCTT TCTTCTGATT CTTTCCTTCA	2940
	CTTTAAAATA GAAAGAAAAA AGAAAACAC ACTGATAGCT CCTTCCATTC CCATATCTCC	3000
	CACTTGATTC TCAAAAACAC ATTTCTCCAA AATAATTGTG TATATGGCGA CAACAACCCA	3060
35	TGAAAGCGAT CTCCAATCTC CAATTATTCA CTCCTCCATC TCCATTATA CATTAACCCC	3120
	TCAACCTTAA CTCTTCACTT CCACACTCCA TTTTCATGGC GACCGACGCC ACTCACCCCTG	3180

WO 97/17452

PCT/EP96/04807

AATTTCTCCA CGTACCAAAA CCTAACCTC ATGAATTCCA CCCAGAAATC TCTATCGCGC 3240

CGTCGCATGA TGGCCTTCAG TTCTGGCAGT TCATGATCGC CGGTTCAATC GCTGGATCAA 3300

5 TCGAGCATAT GGCGATGTAT CCGGTTGATA CGCTTAAAC TCGCATAACAG GGTATTGGGT 3360

CATGTTCCGC TCAATCCGCC GGTCTCCGAC AAGCCCTGG GTCGATACTG AAAGTTGAAG 3420

GTCCCGCCGG ACTTTACCGT GGCATTGGTG CAATGGGTCT CGGTGCAGGA CCAGCTCACG 3480

10 CAGTGTATTT CTCCGTTTAC GAGATGTGTA AGGAGACTTT TTCTCATGGT GATCCGAGCA 3540

ATTCCGGTGC GCACGCCGTT TCGGGGGTGT TCGCGACGGT GGCAAGCGAC GCGGTGATTA 3600

15 CGCCGATGGA TGTGGTGAAA CAGAGGTTGC AGTTGCAGAG CAGTCGTAC AAGGGTGTG 3660

TTGATTGCGT GAGGAGGGTG TTGGTAGAAG AAGGGATTGG CGCATTTCAC GCATCTTATC 3720

GAACAACTGT GGTCACTGAAT GCCCCGTTA CGGCCGTTCA CTTGCCACCA TATGAAGCCA 3780

20 CGAAGAAAGG GTTGTGGAG GTGTCGCCGG AGACTGCGAA CGATGAGAAT TTGTTAGTGC 3840

ATGCTACTGC TGGTGCTGCT GCTGGAGCTT TGGCTGCAGT AGTAACCACT CCACTAGATG 3900

25 TTGTCAAAAC TCAGTTGCAG TGCCAAGTAA GTCCCTTTA ACTTTGCACT AAAAAAAA 3960

TAAGATTCAC TGTTCTAATT TCAGAATTAC ACCAATAAAA AAGGACAGAG CTAGCAATGA 4020

CTTGATTCTC TGAATTGCA ATACGATAAT TCAGTATTGA TAGCTTATAG TATGTGGCCA 4080

30 AGCCAAGGCG TAGGATGAAT TTACCAGCCA GTTTGGAAAGT TAATATCTT TTTTGTATGG 4140

AGATATCGAT GAAGTTGGTG TGATTTTGAGT AGTCACTAAA TGAGCTGCTA TCGCATGATA 4200

35 TATTGATGTG TAAAAATATT GAAAAGTGAA AAACGTTCC AGAGAAACAA GCAACTCATC 4260

TTTATTCTTT AGAGATGGAG CTCGATTATG ATATGAACCTT TGAAGCTTTG AATTGATCGA 4320

TGAAGCAACA AGACAAAATC TTTTATATTA AAAAAGTTGT CTTCTGGTG GTTTATTCA 4380

	GGTGTGCG GATGCGACAG ATTTCTAGC AGTCGATTC AGGATGTTAT AGGAAGCATA	4440
	GTGAAGAAAA ATGGATATGT CGGGTTAATG AGGGGGTGGA TTCCCAGAAT GCTATTCAT	4500
5	GCTCCTGCTG CAGCAATCTG CTGGTCTACT TATGAAGCCT CCAAAACATT CTTTCAAAAA	4560
	CTCAATGAGA GCAATAGCAA CAGCTCAGTT ACCTAAGATT TCATATGTTT TTGTTGTCTC	4620
10	TACTAGGCTT ATCCAAAATC ATGTCGATTG GTTTCACTTC ACCACAGTTG CCATGAACAA	4680
	CTCAAAGCAT CGAATTTCAC ATGTATATTA TGCAATCTAG ATGCTTCTTG ATATTTATTT	4740
	TTATTTTTTC TTTTCCAACT TTTGTAATTA GAATTAGCTA CTATGGTTAT GGCATGGAGT	4800
15	GTGTTATAAT TGCTAATATC ATCGTATAAG CAATGCTATT TGAGAAATTG TGGTGTAAAGG	4860
	TTAGAGTAAT GTTATTTGCC AATCCACTTA CATAGACCGC GGGACTCATT TATCATATGG	4920
20	ACCTACTTCT ATTTCTTATT AGGCAACTAG ATTCTACAAA TAACATTCTC CCGAAGGCTA	4980
	TGTACAATGC ACCTTTTTG AATTACAAAC TCTTCTGTTTC AATATAAGAG GAATCTGGAA	5040
	ATATCTGGTC CTAATTAAC ACAAGTCTAC AAGAATCATG TCATGCCATT AAGGTTCACT	5100
25	TCAAGTAAAG GTGAACACAA ATTAGGAGAA ATTTTAAATT AGAGACACTA	5150

(2) INFORMATION FOR SEQ ID NO: 15:

30 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 328 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: unknown
- (D) TOPOLOGY: unknown

35 (ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

5 (vi) ORIGINAL SOURCE:

(A) ORGANISM: Ribes nigrum

(B) STRAIN: Ben Alder

10

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

Met Ala Thr Asp Ala Thr His Pro Glu Phe Leu His Val Pro Lys Pro

1 5 10 15

15

Lys Pro His Glu Phe His Pro Glu Ile Ser Ile Ala Pro Ser His Asp

20 25 30

20

Gly Leu Gln Phe Trp Gln Phe Met Ile Ala Gly Ser Ile Ala Gly Ser

35 40 45

Ile Glu His Met Ala Met Tyr Pro Val Asp Thr Leu Lys Thr Arg Ile

50 55 60

25

Gln Gly Ile Gly Ser Cys Ser Ala Gln Ser Ala Gly Leu Arg Gln Ala

65 70 75 80

Leu Gly Ser Ile Leu Lys Val Glu Gly Pro Ala Gly Leu Tyr Arg Gly

85 90 95

30

Ile Gly Ala Met Gly Leu Gly Ala Gly Pro Ala His Ala Val Tyr Phe

100 105 110

35

Ser Val Tyr Glu Met Cys Lys Glu Thr Phe Ser His Gly Asp Pro Ser

115 120 125

Asn Ser Gly Ala His Ala Val Ser Gly Val Phe Ala Thr Val Ala Ser

130 135 140

WO 97/17452

PCT/EP96/04807

	Asp Ala Val Ile Thr Pro Met Asp Val Val Lys Gln Arg Leu Gln Leu			
	145	150	155	160
	Gln Ser Ser Pro Tyr Lys Gly Val Val Asp Cys Val Arg Arg Val Leu			
5		165	170	175
	Val Glu Glu Gly Ile Gly Ala Phe Tyr Ala Ser Tyr Arg Thr Thr Val			
	180	185	190	
10	Val Met Asn Ala Pro Phe Thr Ala Val His Phe Ala Thr Tyr Glu Ala			
	195	200	205	
	Thr Lys Lys Gly Leu Leu Glu Val Ser Pro Glu Thr Ala Asn Asp Glu			
	210	215	220	
15	Asn Leu Leu Val His Ala Thr Ala Gly Ala Ala Gly Ala Leu Ala			
	225	230	235	240
	Ala Val Val Thr Thr Pro Leu Asp Val Val Lys Thr Gln Leu Gln Cys			
20	245	250	255	
	Gln Gly Val Cys Gly Cys Asp Arg Phe Ser Ser Ser Ile Gln Asp			
	260	265	270	
25	Val Ile Gly Ser Ile Val Lys Lys Asn Gly Tyr Val Gly Leu Met Arg			
	275	280	285	
	Gly Trp Ile Pro Arg Met Leu Phe His Ala Pro Ala Ala Ile Cys			
	290	295	300	
30	Trp Ser Thr Tyr Glu Ala Ser Lys Thr Phe Phe Gln Lys Leu Asn Glu			
	305	310	315	320
	Ser Asn Ser Asn Ser Val Thr			
35	325			